The mitochondrial fission proteins Fis1 and Drp1 are important for proper glucose-induced insulin secretion in INS1 832/13 cells

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Background and aims: Mitochondria in living cells exist as a dynamic network that continuously cycle through fusion-fission events. Dysfunctional parts of the network were sorted out by mitophagy to support mitochondrial vitality. Mitofusin 1 and 2 (Mfn1 and Mfn2) and the optic atrophy 1 (Opa1) are essential for mitochondrial fusion, whereas the fission protein 1 (Fis1) and the dynamin related protein 1 (Drp1) control fission. It is currently being discussed if both fission proteins, Fis1 and Drp1, are essential to maintain mitochondrial function and glucose-induced insulin secretion of pancreatic beta cells. Thus, the aim of this study was to investigate changes in mitochondrial dynamics and cellular function after knockdown of Fis1 and Drp1 in glucose-responsive INS1 832/13 cells.

Materials and methods: Down-regulation of Fis1 and Drp1 were achieved using the GIPZ Lentiviral short hairpin RNAmir expression system. INS1 832/13 cells were infected with lentiviral particles containing shRNA for Fis1 or Drp1, or a non-silencing shRNA as control. Gene and protein expression were analysed by Real-Time PCR, western blot, and immunofluorescence analyses, respectively. The ATP content was measured using the ATPlite assay. Glucose-induced insulin secretion was determined by ELISA. Mitochondrial morphology and membrane potential were determined by MitoTracker and TMRE staining.

Results: Knockdown of Fis1 and Drp1 in INS1 823/13 cells resulted in a significantly reduced gene expression compared to control cells. Immunofluorescence and western blot analyses showed that Fis1 and Drp1 protein expression was likewise decreased. The mitochondrial membrane potential was significantly reduced in shFis1 and shDrp1 cells compared to control cells by 40 and 20 %, respectively. We observed a homogenous mitochondrial network structure in INS1 823/13 control cells. Knockdown of Fis1 and Drp1 resulted in a significantly higher mean mitochondrial area compared to control cells. However, whereas the Fis1 knockdown especially increased the value of elongated mitochondria in INS1 823/13 cells, after Drp1 knockdown also a number of clumped mitochondria were detectable. The ATP content and glucose-induced insulin secretion was significantly reduced, both in shFis1 and shDrp1 cells compared to control INS1 823/13 cells.

Conclusion: Our results suggest that both proteins, Fis1 and Drp1 are important for the stimulus secretion coupling in INS1 823/13 cells. Because the reduced expression of Drp1 and Fis1 evoked a different mitochondrial morphology, we propose for both proteins independent mechanisms on mitochondrial dynamics and cellular function in pancreatic beta cells.

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